

| <u>Set Name</u><br>side by side                          | <u>Query</u>                   | <u>Hit</u><br><u>Count</u> | <u>Set</u><br><u>Name</u><br>result set |
|--|--------------------------------|----------------------------|---|
| <i>DB=USPT,JPAB,EPAB,DWPI,TDBD; PLUR=YES;<br/>OP=ADJ</i> |                                |                            |   |
| <u>L13</u>   | L12 not l10                    | 0                          | <u>L13</u>                              |
| <u>L12</u>   | l5 and l11                     | 7                          | <u>L12</u>                              |
| <u>L11</u>   | (bromelin or ficin)            | 1121                       | <u>L11</u>                              |
| <u>L10</u>   | l5 and l7                      | 16                         | <u>L10</u>                              |
| <u>L9</u>  | l6 with L8                     | 1120                       | <u>L9</u>                               |
| <u>L8</u>  | plant                          | 499134                     | <u>L8</u>                               |
| <u>L7</u>  | (papain or bromelain)          | 6030                       | <u>L7</u>                               |
| <u>L6</u>  | (protease or proteolytic)      | 52355                      | <u>L6</u>                               |
| <u>L5</u>  | l3 and l4                      | 199                        | <u>L5</u>                               |
| <u>L4</u>  | hydroly\$                      | 279154                     | <u>L4</u>                               |
| <u>L3</u>  | l1 same L2                     | 528                        | <u>L3</u>                               |
| <u>L2</u>  | defatted                       | 6911                       | <u>L2</u>                               |
| <u>L1</u>  | (soy or soya or soybean) flour | 3769                       | <u>L1</u>                               |

END OF SEARCH HISTORY



Generate Collection

L10: Entry 13 of 16

File: USPT

Mar 29, 1977

DOCUMENT-IDENTIFIER: US 4015019 A

TITLE: Preparation of foaming soybean products and the products therefrom

Abstract Paragraph Left (1):

Foaming soybean products useful in food industries can be prepared through series of steps (1) subjecting soybean materials to enzymatic partial hydrolysis without application of any treatment for removing soybean whey component, (2) heating the hydrolyzed product for a short time to inactivate the enzyme, (3) cooling the heated product at neutral or weakly acid pH, (4) removing the precipitates formed, and (5) concentrating the resultant solution followed by pulverization. The product is producible in a high yield from soybean meal with less industrial wastes to be disposed, and has good foaming property.

Brief Summary Paragraph Right (5):

From the circumstances, it has been noticed to utilize soybean protein which is rather cheap and has relatively small fluctuations in price, and many methods of preparation of foaming products by subjecting soybean protein to enzymatic partial hydrolysis with proteolytic enzymes have been reported.

Brief Summary Paragraph Right (13):

In the process of this invention, soybean materials which contain whey component as well as soybean protein are employed as the starting material. As such soybean material, there may be exemplified whole soybean, decoated soybean, ground soybean, soybean flakes, coarsely ground soybean, defatted soybean and so on. These may conveniently be used in powdery form or in flake form. Among them, it is specifically recommendable to use defatted soybean flour or defatted soybean flake which is readily subjected to enzymatic hydrolysis treatment.

Brief Summary Paragraph Right (14):

The first step of the process of this invention is an enzymatic partial hydrolysis of such soybean material as above recited. The enzymatic partial hydrolysis is carried out advantageously in an aqueous dispersion, suspension or solution. A water proportion of which being about 5 to 50 times the amount (weight) of the soybean material, preferably about 10 to 20 times on the same basis.

Brief Summary Paragraph Right (17):

As the aforementioned proteolytic enzyme, acid, neutral or alkaline protease may be used, and it may be of the vegetable, animal or microbial origin. For example, pepsin, papain, acid protease obtained by the cultivation of the microorganism belonging to the family, Polyporaceae, for example *Trametes sanguinea* (L. ex Fr.) Llord, *Trametes cinnabarin* (Jacq.) Fr., *Poria vaporaria* (Fr. non Pers.) Cooke, etc. may be used. The proteolytic enzyme is added to the aqueous dispersion, suspension or solution and the enzymatic hydrolysis may preferably be carried out under optimal pH and temperature in accordance with the proteolytic enzyme used with conventional manner until the partially hydrolyzed product has attained an analytical value of 0.4 to 0.7, preferably of 0.5 to 0.6.

Brief Summary Paragraph Right (18):

Hereinafter, the method of computing the analytical value showing the progress of enzymatic hydrolysis is described.

Brief Summary Paragraph Right (19):

The partially hydrolyzed product to be assayed, which has been derived from the soybean material, is heated and, after the internal temperature has reached 90.degree. C, the heated product is maintained at that temperature for 3 minutes. Then, the heated product is cooled to room temperature (about 20.degree. C) and, then, diluted with water so that the nitrogen content of the system will be 0.1 weight percent. Then, to 2 ml. of the resulting dilution is added 8 ml. of a protein precipitant (a mixture of 0.05M trichloroacetic acid, 0.10M sodium acetate and 0.10M acetic acid) and while the system is maintained at 30.degree. C, it is allowed to stand for 30 minutes. The mixture is filtered and 5 ml. of 0.5M sodium carbonate and 1 ml. of a 2 fold dilution of phenol reagent (Folin's reagent) (The procedure for preparation of the phenol reagent is described in the J. Biol. Chem. 73 629 (1927)) to 2 ml. of the filtrate.

Brief Summary Paragraph Right (21):

As a control, the enzymatically untreated product derived from the same soybean material as used in the above was also treated in the same manner as above and its absorbance at 660 m.mu. is measured. The value found is subtracted from the absorbance value of the enzymatically treated system and the balance is taken as the analytical value of the enzymatically hydrolyzed product.

Brief Summary Paragraph Right (22):

It should be mentioned that among the afore-recited proteolytic enzymes, acid proteases, in particular, have the advantages that the resulting product is of superior quality and that spoilage in the production process may be inhibited. Since pepsin is generally one of the most common acid proteases, the application of this enzyme will be described in detail. And, in case of using other acid proteases, the hydrolysis may similarly be carried out by modifying the method of using pepsin being described below while taking into consideration the properties of the acid protease used, i.e. optimal pH and temperature and so on. Namely, for example, about ten times the weight of defatted soybean flour of water is added and the mixture is stirred evenly to prepare a suspension of soybean flour.

Brief Summary Paragraph Right (23):

In carrying out this operation, the temperature of the system is preferably in the range of 30.degree. to 70.degree. C and, for still better results, about 35.degree. to 60.degree. C. The pH of the system is preferably in the range of pH about 1.5 to 3.5, preferably pH about 2 to 3. Then, pepsin with a protease activity of 100 thousand units/g. (The protease activity is a value determined by the modified Anson-Hagiwara's method, which is described in Kosa Kenkyu Ho (method for Enzyme Studies) II, edited by Shiro Akabori et al., published by Kabushiki Kaisha Asakurashoten (1956) P 240, and this method is in detail described hereinafter; the same definition applies hereinafter) is added to the above soybean flour suspension in a proportion of about 0.1 to 1 weight percent, preferably about 0.5 weight percent based on the weight of proteins contained in the soybean flour suspension, and the enzymatic hydrolysis is carried out for about 30 minutes to 30 hours, preferably for about 1 to 20 hours while the system is maintained at the aforementioned temperature.

Brief Summary Paragraph Right (27):

The enzymatic hydrolysis is terminated when the hydrolyzed product has attained an analytical value of 0.4 to 0.7, preferably 0.5 to 0.6. If the analytical value is out of this range, there may possibly be expected some disadvantageous results. Namely, when the analytical value is less than 0.4, the resulting product does not necessarily display adequate foam expansion and, because of the increased amounts of insolubles that are separated and removed, the product yield is sacrificed to some extent. On the other hand, when the analytical value is higher than 0.7, the resulting product is generally inferior in the shelf life of foam and in most cases has a bitter taste. (cf. Experiment 3 hereinafter).

Brief Summary Paragraph Right (28):

The partially hydrolyzed product thus obtained is further subjected to a combination treatment of (A) heating at an elevated temperature for a short time, (B) cooling and, if necessary, (C) pH adjustment, whereby a product whose temperature is not higher than 60.degree. C with a pH value of 5 to 7 is prepared.

Brief Summary Paragraph Right (29):

The aforementioned heating at an elevated temperature for a short time means an operation that the partially hydrolyzed product is heated and, after the product has attained a temperature of about 90.degree. C, it is maintained at this temperature for about 1 to 10 minutes, preferably for 3 to 5 minutes. The aforementioned cooling is an operation in which the heated product is cooled to an internal temperature of not more than 60.degree. C, preferably between room temperature and 60.degree. C.

Brief Summary Paragraph Right (30):

The aforementioned pH adjustment is an operation to be conducted upon necessity, whereby pH of the product is brought to 5-7. This pH adjustment may be carried out, if necessary, at any stage during the step in which the partially hydrolyzed product is subjected to the combination treatment which may be carried out either before or after the heat treatment, or either before or after the cooling step.

Brief Summary Paragraph Right (31):

If, however, the partial hydrolyzate has a pH value within such a range, this step is naturally unnecessary.

Brief Summary Paragraph Right (33):

Moreover, the combination treatment may include a procedure of removing insolubles which may occur in the system. That is, heating or cooling process of the partial hydrolyzate generally causes insolubles which are preferably removed in the course of the combination treatment at the optional stage on necessity. To explain more concretely, an example of the incorporation of such removing treatment into the combination treatment will be disclosed hereunder.

Brief Summary Paragraph Right (34):

A preliminary (first) separation step may be applied to cooled product obtained by cooling treatment, at the internal temperature of not higher than 60.degree. C, preferably at a temperature between room temperature and 60.degree. C to remove insolubles. The supernatant fluid thus prepared is heated at a temperature of not less than about 70.degree. C, preferably about 80.degree. C to 95.degree. C, for not more than about 30 minutes, preferably about 10 to 20 minutes and, thereafter, the fluid is cooled to an internal temperature of not higher than 60.degree. C, preferably from room temperature to 60.degree. C. Then, the cooled product is subjected to principal (second) separation step. When such a first and a second separation procedures are followed, pH adjustment may be carried out, if required, at any stage of this combination treatment including the case in which the pH adjustment is carried out before or/and after the preliminary (first) separation procedure. Thus, the partially hydrolyzed product is changed into a conditioned preparation with a pH value of 5 to 7 and an internal temperature of not higher than 60.degree. C, preferably from room temperature to 60.degree. C, at a stage prior to finally securing the aforementioned filtrate by removal of insolubles. And a further improvement in product quality can be accomplished by carrying out these first and second separation procedures (cf. Examples hereinafter).

#### Brief Summary Paragraph Table (1):

Table 1

| Conventional processes (I)  | (II)                    | (Modified process of that) | (Modified process of that described in U.S. Patent No.2,489,173) | (Modified process of that described in U.S. Patent No.2,489,208)  |
|-----------------------------|-------------------------|----------------------------|--|---|
| Defatted soybean flakes     | Defatted soybean flakes | Defatted soybean flakes    | Defatted soybean flakes  | Defatted soybean flakes   |
| ##STR1##                    | ##STR2##                | Suspension in acid         | Suspension in neutral or aqueous solution weakly basic           | ##STR3##  |
| ##STR4##                    | Removal of soybean whey | Removal of insolubles      | ##STR5##   | ##STR6##  |
| Enzymatic <u>hydrolysis</u> | pH adjustment           | ##STR7##                   | ##STR8##   | Addition of peptizing salt  |
| Removal of soybean whey     | ##STR9##                | ##STR10##                  | pH adjustment  | Enzymatic <u>hydrolysis</u>   |
| ##STR11##                   | ##STR12##               | Removal of insolubles      | Deactivation of protease   | ##STR13##   |
| ##STR14##                   | pH adjustment           | pH adjustment              | ##STR15##  | ##STR16##   |
| Concentration               | Concentration           | ##STR17##                  | ##STR18##  | Drying  |
| ##STR19##                   | ##STR20##               | ##STR21##                  | ##STR22##  | (note) Insolubles marked with asterisk(*) is known as soybean curd-refuse which is mainly composed of cellulosic substances with a little amount of proteinous, fatty substances and so on. |

#### Brief Summary Paragraph Table (2):

- Process of this invention Defatted soybean (flakes) Suspension in aqueous solution Partial enzymatic hydrolysis with proteolytic enzyme ##STR23## ##STR24## Removal of insolubles ##STR25## ##STR26## ##STR27## Product with a pH of 5-7 whose temperature being not higher than 60.degree. C ##STR28## ##STR29## ##STR30## Drying ##STR31## ##STR32## The foaming soybean products of this invention contain as high as about 30 weight % of soybean whey component relative to the whole quantity. Compared with the product of the present invention, the products obtainable by a conventional process contains about 3 weight % of soybean

#### Detailed Description Paragraph Right (2):

The conventional processes for producing a foaming agent based on soybean protein invariably involve an extractive removal of the soybean whey component prior to an enzymatic hydrolysis. The following is an example.

#### Detailed Description Paragraph Right (3):

To 90 kg. of water was added 550 g. of concentrated hydrochloric acid, followed by the addition of 10 kg. of defatted soybean flour. After the system was adjusted to pH 4.5, it was gently stirred at room temperature for about 1 hour to extract the acid-soluble fraction contained in the soybean material. Then, the system was centrifuged to remove the soybean whey component and the supernatant fluid was discarded. To the residue was added 70 kg. of water and, after stirring for 30 minutes, the mixture was centrifuged. The supernatant fluid was discarded. To 43 kg. of the residue thus obtained was added 35 kg. of lukewarm water (40.degree. C) to evenly disperse the same. The system was adjusted to pH 2 with concentrated hydrochloric acid, its temperature being brought to 40.degree. C. 16 g. of pepsin with a protease activity of 100,000 units/g. was dissolved in a small amount of water and the resulting solution was added to the above system. While the above internal temperature was maintained, the system was gently stirred to carry out the enzymatic hydrolysis, for 20 hours. In the enzymatically hydrolyzed product thus obtained was dissolved 150 g. of sodium chloride and, then, the pH of the mixture was brought to 3.5 by gradual addition of aqueous sodium hydroxide. The mixture was left standing for a while, after which the insolubles in the slurry were removed by centrifugation. Using a vacuum concentrator, the filtrate thus obtained was concentrated at an internal temperature of 60.degree. C to recover 14 kg. of a concentrate with a solids content of about 20%. This concentrate was then spray-dried with a spray dryer of the disc type. The procedure yielded 2.7 kg. of a soybean product (hereinafter referred to as "prior-art product I"), as white powder with a moisture content of 5.8%.

#### Detailed Description Paragraph Right (5):

While the mixture was maintained at an internal temperature of about 50.degree. C, it was gently stirred for about 1 hour, after which the insolubles were removed by centrifugation. The resulting aqueous extract was adjusted to pH 4.5 with 10% hydrochloric acid to coagulate and precipitate the soybean protein curd. The soluble fraction was then removed by centrifugation to remove the soybean whey component. To 7.5 kg. of thus-obtained soybean protein curd were added sufficient quantities of lukewarm water and concentrated hydrochloric acid to prepare a slurry with a solids content of 5 weight %, pH 2 and an internal temperature of 40.degree. C. Separately, 10 g. of pepsin (protease activity 100,000 units/g.) was dissolved in a small amount of

water. The pepsin solution thus obtained was added to the above slurry and the enzymatic hydrolysis was carried out for 4 hours, with gentle stirring and while the above internal temperature was maintained.

Detailed Description Paragraph Right (6):

Then, the enzymatically hydrolyzed product was heated to an internal temperature of 75.degree. C, at which level it was maintained for 30 minutes. After cooling to room temperature, an aqueous solution of sodium hydroxide (30% concentration) was gradually added under stirring until the pH became 6.5. The slurry was then spray-dried to recover 2.5 kg. of a soybean product (hereinafter referred to as "prior-art product II") as white powder with a moisture content of 5.7%.

Detailed Description Paragraph Right (7):

To 2.5 kg. of defatted soybean flour was added 10 kg. of lukewarm water (about 40.degree. C) and, after stirring to homogeneity, the mixture was adjusted to pH 2 with concentrated hydrochloric acid and an internal temperature of about 40.degree. C.

Detailed Description Paragraph Right (8):

Then, a solution of 5 g. of pepsin (protease activity 100,000 units/g.) in a small amount of water was added to the above mixture. The enzymatic hydrolysis was carried out for 5 hours, with gentle stirring and while the above internal temperature was maintained. The procedure yielded a partially hydrolyzed product with an analytical value of 0.54.

Detailed Description Paragraph Right (9):

The hydrolyzed product thus obtained was divided into five aliquot portions and each portion was heated and cooled under the conditions set forth below in the table II. Then, each processed soybean product was adjusted to pH 5 by gradual addition of an aqueous solution of sodium hydroxide and centrifuged to remove the insolubles. The filtrate was concentrated at an internal temperature of 60.degree. C with a vacuum concentrator to obtain a concentrate with a solids content of about 20%. The concentrate was spray-dried to obtain a foaming soybean product sample (1). A test for foaming property and a test for flavor of the foam formed were performed on samples prepared in the above manner.

Detailed Description Paragraph Right (15):

The test results are set forth below in the table II. It was clearly demonstrated that a soybean produced with an excellent flavor and excellent foaming property can be obtained by subjecting the enzymatically hydrolyzed product to a heating at an internal temperature of 90.degree. C for 1-10 minutes and a subsequent cooling to 30.degree. C.

Detailed Description Paragraph Right (16):

By a procedure similar to that described in Experiment 1, defatted soybean flour was subjected to enzymatic hydrolysis using pepsin (100,000 units/g.) to obtain an enzymatically hydrolyzed product with an analytical value of 0.53. This hydrolyzed product was heated to 90.degree. C and maintained at the same temperature for 3 minutes. Then, it was cooled to room temperature (about 20.degree. C). The cooled product was divided into equal portions and each aliquot was adjusted to the pH indicated in the following table and centrifuged to remove the insolubles. Using a vacuum concentrator, each supernatant fluid was concentrated at an internal temperature of 60.degree. C, whereby a concentrate with a solids content of about 20% was obtained. The concentrate was spray-dried to recover a foaming soybean product sample (2) and a test for foaming property and a test for condition and flavor of the foam formed from the sample were carried out on the sample by the testing procedures set forth in Experiment 1.

Detailed Description Paragraph Right (17):

The test results are shown below in the table III. It has been clearly demonstrated that a product with significantly superior flavor, the foaming property, and condition of the foam can be obtained by first preparing a slurry with a pH value of 5 to 7 and then, removing the undesirable factors occurring in minor proportions in the soybean whey component and protein hydrolysate practically as the insolubles are removed by filtration.

Detailed Description Paragraph Right (18):

To 4 kg. of extracted-soybean flour was added 40 kg. of lukewarm water (about 40.degree. C) and, after stirring to homogeneity, the mixture was adjusted to pH 2 with concentrated hydrochloric acid, the internal temperature of the mixture being brought to 37.degree. C. The mixture was divided into 8 equal portions and each portion was subjected to the enzymatic hydrolysis using pepsin (100,000 units/g.) until it had shown the analytical value indicated below in the table IV. Each portion was heated to an internal temperature of 90.degree. C, after which it was maintained at the same temperature for 3 minutes, followed by cooling to room temperature (about 20.degree. C). Then, while each hydrolyzed product was stirred, it was adjusted to pH 5.5 by the gradual addition of an aqueous solution of sodium hydroxide. The slurry was centrifuged to remove the insolubles and the resulting filtrate was concentrated at an internal temperature of 60.degree. C with a vacuum concentrator. The resulting concentrate with a solid content of 20% was spray-dried to obtain a foaming soybean product sample (3).

Detailed Description Paragraph Right (20):

The test results are set forth below in the table IV. It has been clearly demonstrated that an improved result can be obtained by carrying out the enzymatic hydrolysis until analytical value of 0.4 to 0.7.

Detailed Description Paragraph Right (22):

90 kg. of lukewarm water (about 40.degree. C) was added to 10 kg. of the same defatted soybean flour as that used in Reference Example 1 and, after stirring to homogeneity, the mixture was adjusted to pH about 2 with concentrated hydrochloric acid, the temperature of the mixture being brought to about 40.degree. C. To this mixture was added a solution of 18 g. of pepsin (protease activity: 100,000 units/g.) in a small quantity of water and while the system was gently stirred at the above internal temperature for 4 hours. By this enzymatic hydrolysis was obtained an enzymatically hydrolyzed product which showed an analytical value of 0.53. This slurry was heated to an internal temperature of 90.degree. C, at which level it was maintained for 3 minutes, followed by cooling to room temperature. The cooled slurry was adjusted to pH 5 by the gradual addition of aqueous sodium hydroxide (30% concentration) with stirring and, then, the thus obtained conditioned preparation with pH 5 was allowed to stand for a while. It was then centrifuged to remove the insolubles and 75 kg. of the resulting supernatant fluid was concentrated at an internal temperature of 60.degree. C with a vacuum concentrator to obtain 32 kg. of a concentrate with a solids content of about 20%. This concentrate was spray-dried to recover 6.3 kg. of a soybean product (hereinafter referred to as "product A of this invention") as a white powder having a moisture content of 5.6%.

Detailed Description Paragraph Right (23):

A conditioned preparation with pH 5 which was obtained by subjecting soybean to an enzymatic hydrolysis, heating, cooling, pH adjustment and other procedure similar to the foregoing was centrifuged (preliminary separation) to obtain 75 kg. of a supernatant fluid (pH 5). This supernatant fluid was heated again to 90.degree. C, maintained at the same temperature for 10 minutes and cooled to 50.degree. C. The insolubles were then removed by centrifugation (second separation). The resulting supernatant fluid was concentrated at an internal temperature of 60.degree. C with a vacuum concentrator to obtain 32 kg. of a concentrate with a solids content of about 20 %. This concentrate was spray-dried to recover 6.1 kg. of a soybean product (hereinafter referred to as "product B of this invention") as a white powder showing a moisture content of 5.7 %.

Detailed Description Paragraph Right (24):

An enzymatically hydrolyzed product of pH 2, prepared in the same manner as above, was similarly heated, cooled and centrifuged (preliminary separation) to obtain 75 kg. of a supernatant fluid (pH 2). The supernatant fluid was adjusted to pH 5.5 by the gradual addition of aqueous sodium hydroxide (30 % concentration) with stirring.

Detailed Description Paragraph Right (37):

90 kg. of lukewarm water (about 40.degree. C) was added to 10 kg. of defatted soybean flour and, after stirring to homogeneity, the mixture was adjusted to pH about 2 with concentrated hydrochloric acid, the temperature of the mixture being brought to about 55.degree. C. To this mixture was added a solution of 18 g. of pepsin (protease activity: 100,000 units/g.) in a small quantity of water and while the system was gently stirred at the above internal temperature for 1 hour. By this enzymatic hydrolysis was obtained an enzymatically hydrolyzed product which showed an analytical value of 0.52. This slurry was heated to an internal temperature of 90.degree. C, at which level it was maintained for 5 minutes, followed by cooling to room temperature. The cooled slurry was centrifuged to obtain a supernatant fluid. This supernatant fluid was adjusted pH 5.5 by the gradual addition of aqueous sodium hydroxide (30 % concentration) with stirring and, then, the fluid was heated to an internal temperature of 90.degree. C, maintained at the same temperature for 15 minutes and cooled to 50.degree. C. The insolubles were then removed by centrifugation. The resulting supernatant fluid was concentrated at an internal temperature of 60.degree. C under reduced pressure to obtain a concentrate with a solid content of about 20 %. This concentrate was spray-dried to recover 6.1 kg. of a soybean product as a white powder (hereinafter referred to as "product D of this invention").

Detailed Description Paragraph Right (39):

To 10 kg. of defatted soybean flour was added 90 kg. of lukewarm water (about 40.degree. C) and, after stirring to homogeneity, the mixture was adjusted to pH 2.5 with concentrated hydrochloric acid. Separately, 40 g. of an acid protease with a protease activity of 50,000 units/g., obtained by the cultivation of *trametes sanguinea* (L. er Fr.) Lloyd and subsequent purification of the culture (cf. U.S. Pat. No. 3,097,145), was dissolved in a small amount of water. This solution was added to the above mixture and the enzymatic hydrolysis was carried out for 10 hours, with gentle stirring and while the above internal temperature was maintained. The procedure yielded an enzymatically hydrolyzed product with an analytical value of 0.55.

Detailed Description Paragraph Right (40):

This hydrolyzed product was heated to an internal temperature of 90.degree. C and maintained at the same temperature for 3 minutes, followed by cooling to room temperature. The cooled slurry was adjusted to pH 5.5 by the gradual addition of aqueous sodium hydroxide (30 % concentration) with constant stirring. The slurry was allowed to stand for a while, after which it was centrifuged to remove the insolubles. 73 kg. of the resulting supernatant fluid was concentrated at an internal temperature of 60.degree. C under reduced pressure to obtain 30 kg. of a concentrate with a solids content of about 20 %. The concentrate was spray-dried to recover 6 kg. of a soybean product (hereinafter referred to as "product A' of this invention") as a white powder with

a moisture content of 6.5 %.

Detailed Description Paragraph Right (41):

A conditioned preparation of pH 5.5, which was obtained by subjecting soybean to the same enzymatic hydrolysis, heating, cooling, pH adjustment, etc. as above, was centrifuged (first separation) to obtain 73 kg. of a supernatant fluid (pH 5.5). This supernatant fluid was heated once to 90.degree. C and maintained at this temperature for 10 minutes. After cooling to 50.degree. C, the insolubles were removed by centrifugation (second separation). Using a vacuum concentrator, the supernatant fluid was concentrated at an internal temperature of 60.degree. C to obtain 30 kg. of a concentrate with a solids content of about 20 %. The concentrate was spray-dried to recover 5.9 kg. of a soybean product (hereinafter referred to as "product B' of this invention") as a white powder with a moisture content of 6.3 %.

Detailed Description Paragraph Right (42):

An enzymatically hydrolyzed product of pH 2.5, obtained in the same manner as above, was heated, cooled and centrifuged (first separation) to obtain 73 kg. of a supernatant fluid (pH 2.5). This supernatant fluid was adjusted to pH 5.5 by the gradual addition of aqueous sodium hydroxide (30 % concentration) with stirring. It was then heated again to 90.degree. C and maintained at this temperature for 10 minutes, after which it was cooled to 50.degree. C. The insolubles were removed by centrifugation (second separation) and the resulting supernatant fluid was concentrated at an internal temperature of 60.degree. C with a vacuum concentrator to obtain 30 kg. of a concentrate with a solids content of about 20 %. This concentrate was spray-dried to recover 5.8 kg. of a soybean product (hereinafter referred to as "product C' of this invention") as a white powder with a moisture content of 6.4 %.

CLAIMS:

1. A method for preparing soybean products having good foaming properties, which comprises:

1. subjecting soybean materials selected from the group of whole soybean, decoated soybean, ground soybean, soybean flakes, coarsely ground soybean and defatted soybean in powdery or flake form containing both whey component and protein to enzymatic hydrolysis, without removing the whey component, until a partially hydrolyzed product, of which the analytical value at 660 m.mu. showing degree of hydrolysis coming within the range of 0.4 to 0.7, is obtained;

2.

2. heating the partially hydrolyzed product at about 90.degree. C for 1-10 minutes, cooling to a temperature no greater than 60.degree. C, the pH of the partially hydrolyzed product being brought to a value from 5 to 7 before or after said heating or cooling step;

3. removing precipitates formed; and

4. concentrating and/or drying the resultant solution. 2. A method according to claim 1, wherein step (2) comprises:

a. heating the partially hydrolyzed product at about 90.degree. C for 1-10 minutes;

b. cooling to a temperature no greater than 60.degree. C;

c. removing insolubles;

d. heating the resultant solution at not lower than 70.degree. C for no longer than 30 minutes;

e. cooling to a temperature no greater than 60.degree. C; and

f. adjusting the pH of the partially hydrolyzed product to a value from 5 to 7 before or after said heating or cooling step.

4. A method according to claim 1, wherein the enzymatic hydrolysis is carried out by using pepsin.



Generate Collection

L10: Entry 4 of 16

File: USPT

Mar 31, 1992

DOCUMENT-IDENTIFIER: US 5100679 A

TITLE: Method of making a modified proteinaceous product and composition thereof

Abstract Paragraph Left (1):

A process and composition thereof for making a proteinaceous product which comprises preparing an aqueous slurry of soy protein; treating the slurry by adjusting the pH to about 3.5 to about 6 and adding a viscosity reducing agent selected from the group consisting of a proteolytic enzyme and carbohydrase enzyme and an antioxidant, or mixtures thereof to form a pretreated slurry; heating the pretreated slurry, to a temperature not greater than 155.degree. C. such that the pretreated slurry does not contain a substantial amount of proteinaceous antinutritional factors and antigenicity factors; treating the pretreated slurry with a hydrolyzing agent from a source of alpha-galactosidase; and drying the proteinaceous material to form a soy product.

Brief Summary Paragraph Right (9):

Several methods for hydrolyzing the flatulence producing sugars have also been suggested. The flatulence producing sugars are those sugars, principally the alpha oligosaccharide, stachyose, raffinose and saccharose, that are not digested in the digestive tract and enter the lower intestine intact where they are anaerobically fermented which results in the production of carbon dioxide, hydrogen and methane. U.S. Pat. Nos. 4,483,874, 4,376,127, 4,216,235 and 3,632,346 describe various enzymatic treatments which are indicated to result in hydrolysis or degradation of the flatulence producing sugars to digestible mono- and di-saccharide sugars.

Brief Summary Paragraph Right (11):

In the treatment process described in U.S. Pat. No. 4,483,874, a crude source of vegetable protein and carbohydrate, for example, full fat or defatted beans or cottonseed, soy flour or meal, is cooked to inactivate trypsin inhibiting factors. The cooked material is slurried in water and contacted with the enzyme at an elevated temperature that is below that at which the enzyme is inactivated, at or slightly below 50.degree. C., the resulting product may be fed directly as a substitute milk source or may be augmented with additional sources of fat, protein and carbohydrate depending on the nutrient content of the starting raw material. Typically, augmentation is required to obtain the nutrient level of mother's milk.

Brief Summary Paragraph Right (19):

The heat treated slurry is then subjected to a hydrolyzing agent. The hydrolyzing agent is alpha-galactosidase. The primary function of which is to hydrolyze the flatulent producing sugars, principally raffinose, stachyose and saccharose to digestible monosaccharides. In a preferred embodiment, the hydrolyzing agent also includes the use of carbohydrase enzyme for further degradation of non-starch polysaccharides and results in further lowering of the slurry viscosity. A protease type of enzyme can also be added to the hydrolyzing agent with or without the carbohydrase enzyme.

Brief Summary Paragraph Right (21):

The treatment of the present invention is described in connection with the use of comminuted vegetable protein and carbohydrates, more specifically, defatted soy flour and soy meal. The defatted soy contains less than 1.2% oil and about 50% protein. Alternatively, defatted rapeseed meal or cottonseed and germ meal may be utilized. Note, all weight percents herein were measured on dry solids.

Brief Summary Paragraph Right (23):

The pretreated slurry is then heated by injection of live steam followed by high shear mixing of the product. The conditions are chosen as such that in the soy slurry the proteinaceous antinutritional factors are greatly reduced or inactivated. Other heating conditions can be employed so long as the antinutritional factors are substantially eliminated. The pretreated slurry is cooled, then treated with a hydrolyzing agent from the source of an alpha-galactosidase which can be combined with carbohydrase or protease individually, or all three components can be used together. Preferably, the hydrolyzing agent is a mixture of alpha-galactosidase and a carbohydrase, and more preferably, the hydrolyzing agent is a mixture of alpha-galactosidase, carbohydrase and a protease. This proteinaceous material is dried to form a protein product.



Brief Summary Paragraph Right (34):

The cooled, pretreated slurry is further treated with a hydrolyzing agent. The hydrolyzing agent is alpha-galactosidase, but could also contain a carbohydrase enzyme and/or a protease enzyme. Preferably, the hydrolyzing agent is a mixture of carbohydrase enzyme and alpha-galactosidase. More preferably, the hydrolyzing agent is a mixture of alpha-galactosidase, carbohydrase and protease enzymes. The standard reaction time is about 4 hours, but can be varied according to the dry solids content of the slurry and the amount of enzymes used. Note, the pretreated slurry should be cooled as stated herein so that when the enzyme mixture is added, the enzymes are not inactivated due to the temperature of the pretreated slurry being too high. The resulting proteinaceous matter has a viscosity of about 500 to about 3500 cps, preferably about 500 to 2500 cps at 50.degree. C., measured by a Spindle Brookfield Viscosimeter.

Brief Summary Paragraph Right (38):

the protease can be from fungal, bacterial and plant extracts and mixtures thereof, or more specifically, fungi from the Aspergillus strain, preferably Aspergillus oryzae; Bacillus strain, preferably Bacillus lichenformis and Bacillus subtilus; and from plant extracts such as papain. A bacterial protease is preferably used with an activity of 0.5AU/g, where AU is Anson units. The more preferred protease is produced from Bacillus subtilus. NEUTRASE 0.5L is the most preferred protease which can be purchased at NOVO Nordisk. Preferably, a bacterial protease is used with an activity of 0.5 AU/gram. The protease is employed to hydrolyze the proteins and to increase the solubility of the final proteinaceous product.

Brief Summary Paragraph Right (39):

The alpha-galactosidase is selected from a group fungal or bacterial alpha-galactosidase or mixtures thereof. Alpha-galactosidase is made by NOVO Nordisk. Alpha-galactosidase can be produced from fungi from the Aspergillus strain, preferably Aspergillus niger and Aspergillus oryzae; the Monascus strain, preferably Monascus pilosus; and from bacteria from the Bacillus stain, preferably Bacillus stearothermophilus. The alpha-galactosidase is preferably of fungal origin (Aspergillus strain) with an activity of 250 GAL unit/gram (GALU is galactosidase units). More preferred is fungal alpha-galactosidase from an Aspergillus niger strain with a minimum activity of 250 GALU/gram. The alpha-galactosidase catalyses the hydrolyses of several sugars such as raffinose, stachyose, and saccharose. When treating the slurry with the enzyme mixture, the temperature is maintained from about 35.degree. C. to about 60.degree. C., preferably about 40.degree. C. to about 45.degree. C. Optionally, the pH of the enzyme/slurry mixture is adjusted to maintain a pH of about 4.0 to about 5.5, preferably about 4.5 to about 5.0, by adding hydrochloric acid, phosphoric acid citric acid, sodium carbonate or sodium hydroxide.

Brief Summary Paragraph Right (40):

When the hydrolyzing agent is just alpha-galactosidase (250 GALU/gram), it is used in an amount of about 0.2 to about 1.3, preferably about 0.4 to about 1.0, more preferably about 0.5 to about 0.8 weight percent. If the alpha-galactosidase has an activity that is higher or lower than 250 GALU/gram, the amount used is adjusted. If the dosage is expressed in GALU/gram dry proteinaceous material for the enzyme treatment, the alpha-galactosidase (250 GALU/gram) is used in an amount of about 0.50 GALU to about 3.25 GALU, preferably about 1.0 GALU to about 2.5 GALU, more preferable 1.25 GALU to about 2.0 GALU.

Brief Summary Paragraph Right (41):

When the hydrolyzing agent contains, in addition to alpha-galactosidase, a carbohydrase enzyme with an activity of 120 FBG/ml, the carbohydrase enzyme is used in an amount of about 0.1 to about 1.0 weight percent, preferably about 0.2 to about 0.85 weight percent. If the carbohydrase enzyme has a lower or higher activity than 120 FBG/ml, the dosage is corrected. If the dosage of the carbohydrase enzymes is expressed in FBG units per gram proteinaceous material for the enzyme treatment, the carbohydrase enzyme is used in an amount of 0.1 to about 1.0 FBG, preferably about 0.2 to about 0.85 FBG units per gram dry product. When the hydrolyzing agent contains a carbohydrase enzyme with an activity of 120 FBG/ml and the alpha-galactosidase has an activity of 250 GALU/gram, the mixture added to the pretreated slurry per gram dry proteinaceous material contains about 1.0 GALU to about 2.0 GALU and 0.15 FBG units to about 1.0 FBG units per gram dry product.

Brief Summary Paragraph Right (42):

The hydrolyzing agent that contains the protease in addition to the alpha-galactosidase and carbohydrase enzymes is used in an amount of about 0.5 to about 2.4 weight percent, preferably about 0.65 to about 2.25 weight percent, whereby the alpha-galactosidase enzyme has an activity of 250 GALU/gram, the carbohydrase enzyme has an activity of 120 FBG/ml and the protease has an activity of 0.5 AU/gram.

Brief Summary Paragraph Right (45):

After forming the proteinaceous matter, it is advisable to pasteurize the matter to make sure that microbial activity is minimized. To pasteurize the proteinaceous matter, the slurry is pumped through a heat exchanger to raise a temperature of about 85.degree. C. for 10 to 20 seconds. If desired, the slurry can be corrected with sodium hydroxide or sodium carbonate or calcium hydroxide to obtain a pH of about 5.0 to about 6.5. The proteinaceous matter is dried by flash drying or spray drying to thereby form a proteinaceous product, wherein spray drying is the preferred technique. When drying the proteinaceous matter, the hydrolyzing agent is a mixture of alpha galactosidase and carbohydrase enzymes. Most preferred, the hydrolyzing agent also contains a

protease. Generally, the proteinaceous matter is dried such that the proteinaceous product contains about 3 to about 11 percent moisture, preferably, about 4 to about 8 percent moisture, most preferably about 4 to about 6 percent moisture based upon the weight of the final proteinaceous product. Additionally, if so desired, but not necessary, amino acids can be added before or after drying the proteinaceous product to further upgrade the product nutritionally, with lysine and/or methionine being preferred.

Brief Summary Paragraph Right (48):

The proteinaceous product is a replacement for standard or normal milk replacers up to about 75 weight percent replacement. More specifically, such a milk replacer contains about 5 to about 30 weight percent proteinaceous product, about 30 to about 50 weight percent whey protein products and about 5 to about 20 weight percent skim milk; more preferably, about 10 to about 25 weight percent proteinaceous product, about 35 to about 45 weight percent whey protein products and about 10 to about 15 weight percent skim milk; most preferred is about 19 weight percent proteinaceous product, about 42 weight percent whey protein products and about 11 weight percent skim milk, with the balance constituting about 20 weight percent fat and the rest being minerals and emulsifiers, where the weight percent is based on the final milk replacer composition. Because the hydrolyzing agent increases the emulsifying capacity of the resulting proteinaceous product, it is possible to add less emulsifier in the substitute milk replacer and, if so desired, either add more minerals or even increase the amount of proteinaceous product used therein. Preferably, the milk replacer also contains about 1 to about 10 weight percent starch or pregelatinized starch, preferably about 4 to about 6 percent based on the weight of the calf milk replacement product.

Brief Summary Paragraph Right (49):

The proteinaceous product can be used in other applications, such as pet food. When employing the proteinaceous product in pet food, it is desirable to make the proteinaceous product such that, when treating the pretreated slurry with the hydrolyzing agent, the mixture comprises the carbohydrase enzyme and alpha-galactosidase. This hydrolyzing agent is preferred since, if the protease is added, the resulting pet food will show reduced texture. In applications for pet food, it is also advised to decant the slurry before the final drying step. When decanted, the amount of lower or reducing sugars in the proteinaceous product will be reduced to levels lower than 8 percent.

Detailed Description Paragraph Right (5):

The following Table 1(ii) illustrates the physical and chemical properties of the proteinaceous product herein, A-A.sub.2, and compares it to that of soy bean concentrate (SBC) and soy bean meal (SBM), where SBC has been treated to remove some carbohydrate and other anti-nutritional factors and is slightly less digestible than milk protein. Milk protein is 92 to 95 percent digestible and SBC is 80 to 85 percent digestible. The problem with use of SBC is that use is cost prohibitive. Soy bean meal is an inexpensive source of protein, but unmodified, it has relatively poor protein digestibility of 63 to 67 percent along with anti-nutritional and antigenic factors that limit its use. Note that the proteinaceous product herein, A and A1 were treated with different enzymatically treatment steps. A was treated with the viscosity reducing agent, a carbohydrase enzyme, and with the hydrolyzing agent containing carbohydrase and protease; where A1 and A2 were pretreated with viscosity reducing agent carbohydrase, but the hydrolyzing agent in the final treatment step also contained an alpha-galactosidase.

Detailed Description Paragraph Right (25):

The soy slurry was then heated using a jetcooking system, which includes the injection of direct steam of 12 bar followed by an intense mixing by an inliner mixer. The process was continuous and the slurry was kept at 125.degree. C. to 135.degree. C. for 5 minutes before cooling in a flashcooler to about 95.degree. C. The slurry was then further cooled to 50.degree. C. by using a tube cooler and pumped to a reaction vessel. The pH of the slurry was 4.9 to 5.0, and did not need to be corrected. The mixture of hydrolyzing enzymes containing an alpha-galactosidase, carbohydrase and protease were added in an amount of 1.62 percent on a dry weight soy product. The enzyme reaction was continuously stirred and at a temperature of about 46.degree. C. to about 49.degree. C. The hydrolyzing agent contained about 30,000 FBG units carbohydrase enzyme, about 96,000 GALU and about 25 AU protease units. The slurry was then spray dried using a spray drier with an atomizer wheel. The inlet temperature for the dryer varied between 190.degree. C. and 210.degree. C. The outlet temperature for the dryer varied between 80.degree. C. and 90.degree. C. The obtained product contained around 6 percent (5.5 to 6.3) moisture and 50 to 52 weight percent protein on a dry basis. The product was analyzed on simple sugars, trypsin inhibitor values and antigenicity as follows:

Detailed Description Paragraph Right (30):

About 230 to about 340 liters of soy slurry were prepared by adding, under continuous stirring, soy flour 200/90 to tap water (50.degree. C.) till a homogeneous suspension was obtained, with a dry solid varying from about 25 to about 27.5 percent. The pH of the slurry was corrected to a pH of about 5.0 by adding 10 percent hydrochloric acid. The viscosity reducing agent carbohydrase agent was added in the range from 0.33 to about 0.87 percent on dry weight basis of the product. The viscosity reducing enzyme was added to hydrolyze the non-starch polysaccharides, which resulted in a lower viscosity before and after the heat treatment. The enzyme reaction proceeded for two hours under continuous stirring. The temperature of the slurry was kept between 42.degree. C. and 48.degree. C. by means of indirect heating. The slurry was then heated by passing it continuously through a jetcooker system which included the in-line mixer. The temperature during the heat treatment was 130.degree. C.-135.degree. C. and the slurry was kept at that temperature for 5-6 minutes before cooling in the flashcooler to about 90.degree. C.

C. The slurry was then further cooled in a tube cooler to about 50.degree. C. and treated with the hydrolyzing agent in the form of a mixture containing a protease, an alpha-galactosidase and a carbohydrase.

Detailed Description Paragraph Right (31):

Dosages of the hydrolyzing mixture varied between 1.19 to about 1.83 weight percent based on dry weight basis of the product. The hydrolyzing agents contained around 7.8 to about 33 AU units protease activity, 30,000 to about 48,000 FBG carbohydrase units and around 112,500 to about 160,000 GALU. The enzyme reaction proceeded for 4 hours under continuous stirring, while the temperature of the mixture was maintained between 42.degree. C. and 51.degree. C. by using indirect heating. The slurry was then spray dried using a spray dryer with inlet temperatures varying between 185.degree. C. and 200.degree. C. and outlet temperatures between 80.degree. C. and 90.degree. C. The obtained dry product was a free flowing powder with a moisture content varying between 4.0 and 6.0 weight percent. The protein content was between 50 and 52 weight percent on dry basis. The product showed an excellent mixability in lukewarm tap water (35.degree. C.-40 .degree. C.) and a 20 weight percent solution remained stable for at least 4 hours. From each run, samples were analyzed for trypsin inhibitor factor, antigenicity and the simple sugars raffinose, stachyose and saccharose. During the process, the viscosities of the slurry were measured using a Brookfield Spindel Viscosimeter at 40.degree. C.

Detailed Description Paragraph Right (34):

The soy slurry was then heat-treated by passing the slurry continuously through a jetcooker (hydroheater M103MSX). Live steam 12.5 Bar was used to heat the slurry to around 150.degree. C. Retention time at that temperature varied between 50 and 80 seconds before the slurry was cooled to 90.degree. C.-95.degree. C. in the flashcooler. The slurry was further cooled to around 50.degree. C. by using a tube cooler. For the final enzyme treatment, a mixture containing hydrolyzing enzymes was added and the reaction did proceed for 4 hours under continuous stirring. The hydrolyzing agent was of the following mixture as described in Table 7 a(i). The temperature was maintained between 40.degree. C. and 51.degree. C. by using indirect heating. The slurry was then spray dried using a standard spray dryer with atomizer wheel. The air inlet temperature was between 190.degree. C. and 200.degree. C. The outlet temperature varied between 75.degree. C. and 90.degree. C. The final dry proteinaceous product was a free-flowing powder with a moisture content between 4 and 6 percent. The protein level varied between 50 and 52 percent. A 20 percent solution of the product in warm tapwater (35.degree. C.) remained stable for at least 4 hours. During the process, the viscosity was measured by using a Brookfield Spindle Viscosimeter. The trypsin inhibitor factor, antigenicity and sugars were analyzed using the described methods.

Detailed Description Paragraph Type 1 (2):

200/20: defatted soy flour with 20 protein dispersibility index (P.D.I.) and a granulation such that 95 percent passes through a screen of 200 mesh.

Detailed Description Paragraph Type 1 (3):

200/90: defatted soy flour with a P.D.I. of 85 and a granulation as above.

Detailed Description Paragraph Type 1 (4):

80/20: defatted soy flour with a P.D.I. of 30 and a granulation as that 95 passes through a screen of 80 mesh.

CLAIMS:

I. A method of making a proteinaceous product by treating sources of vegetable protein and carbohydrates that contain non-starch polysaccharides to improve palatability, digestibility, and minimize proteinaceous antinutritional factors and antigenicity factors, which comprises:

- a) preparing an aqueous slurry of vegetable proteins and carbohydrates;
- b) adjusting the pH of the slurry between about 3.5 and about 6;
- c) pretreating the slurry to reduce the viscosity below about 4000 cps by reacting a viscosity reducing agent with the slurry;
- d) heating the slurry to a temperature between about 85.degree. C. and about 155.degree. C. for a period of time to substantially minimize proteinaceous antinutritional factors and antigenicity factors;
- e) cooling the slurry so that a hydrolyzing agent that is added in step (f) is not inactivated;
- f) hydrolyzing the slurry with a hydrolyzing agent from a source of alpha-galactosidase.

24. The process for making a proteinaceous product of claim I wherein the hydrolyzing agent further contains a carbohydrase

enzyme and the hydrolyzed slurry is spray dried.

25. The process for making a proteinaceous product as in claim 1 wherein the hydrolyzing agent further contains a carbohydrase enzyme.

26. The process for making a proteinaceous product as in claim 1 wherein the hydrolyzing agent further contains protease enzyme.

27. The process for making a proteinaceous product as in claim 26 wherein the hydrolyzing agent is about 0.2 to about 1.3 weight percent alpha-galactosidase with an activity of 250 GALU grams, about 0.1 to about 1.0 weight percent of carbohydrase enzyme with an activity of 120 FBG/ml, about 0.5 to about 2.4 weight percent protease with an activity of 0.5 AU/gram.

40. The proteinaceous product made by the process as in claim 27 wherein the hydrolyzing agent is used in an amount of about 0.5 to about 2.4 weight percent, where the alpha-galactosidase has an activity of 250 GAL units/gram, carbohydrase enzyme has an activity of 120 FBG/ml; protease has an activity of 0.5 AU/gram.

49. A process as in claim 48 which further comprises (g) drying the proteinaceous material by spray drying to form a proteinaceous product, where the hydrolyzing agent used is a mixture of alpha galactosidase, carbohydrase and protease.



## Generate Collection

L10: Entry 14 of 16

File: USPT

Apr 8, 1975

DOCUMENT-IDENTIFIER: US 3876806 A

TITLE: Process for the preparation of acid soluble polypeptides and carbonated beverages containing same

Abstract Paragraph Type 1 (2):

2. controlling the temperature of the heat-treated slurry to provide temperature conditions for enzymatic hydrolysis.

Abstract Paragraph Type 1 (3):

3. subjecting the resulting slurry to enzymatic hydrolysis conditions, including the action of a proteolytic enzyme to produce water-soluble polypeptides,

Brief Summary Paragraph Right (6):

In copending U.S. patent application Ser. No. 165,338, filed July 22, 1971 by Hempenius, Moser, and Valenti, there is disclosed a process of preparing an acidified protein beverage which has the elusive combination of high polypeptide content, good mouth feel, and fairly good taste. This process involves subjecting an aqueous slurry of defatted soya, corn or cotton seed protein to a pre-hydrolysis, denaturation heat treatment, then to the hydrolytic action of a proteolytic enzyme to solubilize the protein, adjusting the pH of the thus treated aqueous slurry to in the range of from about 2.5 to about 6.0, advantageously from about 3.0 to about 4.0 or 4.3, and removing undissolved solids from the slurry to leave a mother liquor which can be formulated into a carbonated beverage.

Brief Summary Paragraph Type 1 (1):

1. heating an aqueous slurry of the defatted soya, corn or cottonseed protein at a temperature of about 150.degree. to 375.degree.F. for a time sufficient to increase the yield of a soluble, nutritional, polypeptide product which is produced when the heated slurry is subsequently subjected to enzymatic hydrolysis, but insufficient to deleteriously affect the flavor of said polypeptide product,

Brief Summary Paragraph Type 1 (2):

2. controlling the temperature of the heattreated slurry to provide temperature conditions for enzymatic hydrolysis.

Brief Summary Paragraph Type 1 (3):

3. subjecting the resulting slurry to enzymatic hydrolysis conditions, including the action of a proteolytic enzyme, to produce water-soluble polypeptides,

Detailed Description Paragraph Right (1):

As stated above, the process of the present invention provides acidic beverages that are rich in dissolved polypeptides obtained from protein provided by one or more of defatted soya, corn, and cotton seeds. By "defatted", when describing the protein that can be used in the process of this invention, is meant protein materials which are substantially free of fat. Because of its low cost and ready adaptability to the process of the present invention, soya protein is generally preferred, and suitable sources thereof include, for example, soya protein isolate, soya flour, soya grits, soya concentrate, soya meal, and their mixtures. It is also preferred that the protein material be ground, powdered, homogenized, comminuted or otherwise suitably converted to small particle size to permit easy and economical dispersion in water at the desired concentration of use.

Detailed Description Paragraph Right (2):

In accordance with the process of the present invention, the pre-hydrolysis, denaturation heat treatment is conducted by heating a slurry of the protein, advantageously an aqueous slurry of defatted soya protein, under denaturation conditions including a temperature and residence time sufficient to substantially destroy vegetative cells, and preferably, but not necessarily, to substantially destroy spores as well. Generally, a temperature of at least about 150.degree.F. for a time sufficient to increase the yield of a soluble, nutritional, polypeptide product, which is produced when the preheated product is subsequently subjected to enzymatic hydrolysis, can be used. The preheat treatment is advantageously conducted in a heating zone under pressure conditions sufficient to maintain the integrity of the slurry, i.e., at a substantially constant volume.

Detailed Description Paragraph Right (4):

So as to optimize the amount of soluble polypeptides obtained from the hydrolysis treatment, the pre-hydrolysis heat treatment is desirably conducted for a relatively long period of time when the temperature employed is relatively low. Conversely, at relatively high treatment temperatures the duration of the denaturation heat treatment is preferably kept short, so as to avoid imparting a burnt taste to the product or substantially degrading the protein.

Detailed Description Paragraph Right (7):

In a particularly advantageous aspect of the present invention, the pre-hydrolysis, denaturation heat treatment can be conducted using soya, preferably in flour or grit form, containing about 40 to 60 wt. percent protein under denaturation temperature and residence time conditions sufficient to provide a nutritional polypeptide product having optimized flavor characteristics as well as being produced in increased yields. In this aspect, the aqueous slurry of defatted soya protein is advantageously heated (a) at a temperature of at least about 200.degree.F., but below a temperature which will deleteriously affect flavor optimization of the soluble polypeptide product produced by subsequent enzymatic hydrolysis, and (b) for a time sufficient to initiate some denaturation, but insufficient to deleteriously affect the flavor optimization of the soluble polypeptide product produced by subsequent enzymatic hydrolysis. Generally, the temperatures for the denaturation treatment, when using soya protein, can range from about 200.degree. to 265.degree.F., preferably from about 215.degree. to 245.degree. or 255.degree.F., and the residence time can often range from about 0.1 to about 30 seconds or more, preferably from about 0.2 to 20 seconds. At higher temperatures, shorter residence times can be used since the desired degree of denaturation can be effected more expeditiously while avoiding the risk of providing a nutritional polypeptide product which is bitter tasting.

Detailed Description Paragraph Right (8):

In the enzymatic hydrolysis phase, the next phase of the process, any suitable proteolytic enzyme which serves to hydrolyze edible protein may be employed in the process of the present invention. Such proteolytic enzymes from animal, vegetable and microbiological sources are well known to the art and include, for example, protease enzymes such as neutral protease, alkaline protease and mixtures thereof, pepsin, ficin, papain, renin, and the like. Enzymes having activity at a pH from about 6.0 to 14.0 are advantageous. However, because of its efficiency of operation and because of its maximum activity at neutral pH (e.g. 6.0 to 7.5), neutral protease is the preferred enzyme for use in the process of the present invention.

Detailed Description Paragraph Right (10):

In carrying out the enzymatic hydrolysis phase of the process of the present invention, the temperature of the denatured protein product from the pre-hydrolysis heat treatment phase is controlled to provide a product having a temperature suitable for the particular enzyme employed. The "controlling" may or may not involve an actual temperature adjustment, e.g., cooling to a temperature suitable for enzyme action. Advantageously, the enzyme employed is one which will normally permit the utilization of the heat-treated product without further modification.

Detailed Description Paragraph Right (11):

The concentration of the denatured protein in the slurry to be subjected to enzyme hydrolysis may vary over a wide range and will depend, among other things, on the particular protein used and the particular enzyme employed. A concentration of protein in the slurry of about 1 to about 15%, based on the total weight of the slurry, is generally suitable.

Detailed Description Paragraph Right (15):

The enzymatic hydrolysis is advantageously conducted at a temperature and residence time sufficient to hydrolyze a predominant amount of the heat-treated protein, for instance at least 65 wt. percent and preferably at least about 75 wt. percent of the protein. The hydrolysis is, from a flavor optimization standpoint, preferably terminated before it proceeds to the point of producing a significant amount of product(s) in addition to the desirable polypeptides which will cause a bitter taste in the mouth of one consuming a beverage containing such polypeptides. A low odor, bland polypeptide product which can be incorporated into beverages is desirable. The hydrolysis is generally conducted to a point, however, to provide a soluble polypeptide product which will not precipitate at a pH below 4.5.

Detailed Description Paragraph Right (16):

Advantageously, the enzymatic hydrolysis can be conducted at a temperature in the range of about 105.degree. to 150.degree.F., preferably 120.degree. to 140.degree.F., and a residence time generally from about 30 minutes (m.) to 150 m., preferably 90 m. to 120 m., particularly when using Montase 110 (DA-10), a commercial enzyme mixture described infra.

Detailed Description Paragraph Right (17):

After the treatment with the enzyme, the pH of the slurry is adjusted to in the range of from about 2.5 to about 6.0. In order to assure that acidified beverages prepared from the resulting solubilized polypeptide are devoid of undesirable precipitate, however, it is particularly advantageous that the pH of the slurry be adjusted to, or below, that which the acidified beverage will have, i.e. up to about 4.0 or 4.3, e.g. about 3.0 to 4.0 or 4.3. When downward pH adjustment is required, as is the usual case, any acid

suitable for food use may be employed. Such acids include phosphoric acid, malic acid, tartaric acid, citric acid, and succinic acid. When the enzymatic hydrolysis is conducted with neutral or alkaline protease such acid treatment can also advantageously serve to inactivate the enzyme. When upward pH adjustment is required, a suitable base for food use such as sodium hydroxide or potassium hydroxide may be employed. The enzyme is preferably inactivated in any event to avoid the production of products having a deleterious affect on the taste of the ultimate product. Thus, if the enzyme is one acting at a pH of 3 to 4.3, for instance, it can be deactivated by heating the slurry to an enzyme deactivating temperature.

Detailed Description Paragraph Right (18):

The whole slurry resulting from the hydrolytic action of a proteolytic enzyme is, advantageously, first treated to adjust the pH of the slurry before any solids are removed. In following this particular aspect, the solids removal during the processing can be conducted more efficiently, while at the same time providing a product with the desired taste characteristics. Thus, it is unnecessary in the present process to subject the slurry to more than one solids separation step. This provides an economic advantage over similar processes which effect removal of undissolved solids both before and after lowering the pH of an aqueous, polypeptide-containing liquor which has been enzymatically hydrolyzed.

Detailed Description Paragraph Right (33):

Example II is repeated in every detail, except that the temperature of the slurry after conclusion of the enzymatic hydrolysis is lowered to about 40.degree., rather than 77.degree.F., prior to separation of precipitated material. This procedure advantageously prevents cryo-precipitation in the finished beverage.

CLAIMS:

1. In the preparation of an acid-soluble polypeptide product for use as a base in preparing acidic, soft drink, protein beverages, by a process comprising the steps of

1. heating an aqueous slurry of defatted protein derived from soya, cotton or corn seeds, under substantially neutral pH conditions, at a temperature of about 150.degree. to 375.degree.F. for a time sufficient to increase the yield of a soluble, nutritional, polypeptide product which is produced when the heated slurry is subsequently subjected to enzymatic hydrolysis, but insufficient to deleteriously affect the flavor of said polypeptide product,

2. controlling the temperature of the heat-treated slurry to provide temperature conditions suitable for enzymatic hydrolysis,

3. subjecting the resulting slurry to enzymatic hydrolysis conditions, including the action of a proteolytic enzyme, to hydrolyze a predominant amount of heat-treated protein and produce water-soluble polypeptides,

4. adjusting the pH of the resulting slurry to about 3.0 to 4.3 and inactivating the enzymes, and

5. removing undissolved solids from the slurry to leave an acidic mother liquor containing dissolved polypeptides; the improvement comprising

6. evaporating from said mother liquor having a pH from about 3.0 to 4.3 substantially all of those off-flavor ingredients in the polypeptide-containing product which boil below the boiling point of water, said evaporation being effected at a temperature sufficient to separate the ingredients from the mother liquor and over a period of time sufficient to evaporate the ingredients but insufficient to effect substantial degradation of the polypeptide in the mother liquor, thus yielding a residue that is suitable for use as a base in preparing acidic, soft drink, protein beverages.

2. In the preparation of an acid-soluble polypeptide product for use as a base in preparing acidic, soft drink, protein beverages, by a process comprising the steps of

1. heating an aqueous slurry of defatted protein derived from soya, cotton or corn seeds, under substantially neutral pH conditions, at a temperature of about 150.degree. to 375.degree.F. for a time sufficient to increase the yield of a soluble, nutritional, polypeptide product which is produced when the heated slurry is subsequently subjected to enzymatic hydrolysis, but insufficient to deleteriously affect the flavor of said polypeptide product,

2. controlling the temperature of the heat-treated slurry to provide temperature conditions suitable for enzymatic hydrolysis,

3. subjecting the resulting slurry to enzymatic hydrolysis conditions, including the action of a proteolytic enzyme having activity at a pH from about 6.0 to 14.0, to hydrolyze a predominant amount of heat-treated protein and produce water-soluble polypeptides,

4. adjusting the pH of the resulting slurry to about 3.0 to 4.3 to provide acidic conditions comparable to the pH desired for the

protein beverage and to inactivate the enzyme, and

14. The process of claim 13 wherein the step (3) hydrolysis is conducted at about 105.degree. to 150.degree.F. for a residence time of about 30 to 150 minutes.

16. The process of claim 15 wherein the step (3) hydrolysis is conducted at about 105.degree. to 150.degree.F. for a residence time of about 30 to 150 minutes.

18. In the preparation of an acidic, carbonated, protein beverage by a process comprising the steps of

1. heating an aqueous slurry of defatted protein derived from soya, cotton or corn seeds under substantially neutral pH conditions at a temperature of about 150.degree. to 375.degree.F. for a time sufficient to increase the yield of a soluble, nutritional, polypeptide product which is produced when the heated slurry is subsequently subjected to enzymatic hydrolysis, but insufficient to deleteriously affect the flavor of said polypeptide product,

2. controlling the temperature of the heat-treated slurry to provide a slurry having a temperature from about 105.degree. to about 140.degree.F. for enzymatic hydrolysis,

3. subjecting the resulting slurry to enzymatic hydrolysis conditions, including the action of a proteolytic enzyme having activity at a pH from about 6.0 to 7.5, to hydrolyze at least 65 wt. percent of the protein and produce water-soluble polypeptides,

4. adjusting the pH of the resulting slurry to about 3.0 to 4.3 to provide acidic conditions comparable to the pH desired for the protein beverage and to inactivate the enzyme, and

5. removing undissolved solids from the slurry to leave an acidic mother liquor containing dissolved polypeptides; the improvement comprising

6. evaporating from said mother liquor having a pH from about 3.0 to 4.3 substantially all of those off-flavor ingredients in the polypeptide-containing product which boil below the boiling point of water, said evaporation being effected at a temperature sufficient to separate the ingredients from the mother liquor and over a period of time sufficient to evaporate the ingredients but insufficient to effect substantial degradation of the polypeptide in the mother liquor, and

7. formulating the evaporation residue into an acidic, carbonated beverage.

27. In the preparation of an acidic, carbonated, protein beverage from an aqueous slurry of defatted soya protein, by a process comprising the steps of

1. heating an aqueous slurry of defatted soya protein in the form of flour or grits under substantially neutral pH conditions at a temperature of about 150.degree. to 375.degree.F. for about 0.01 to 120 seconds,

2. controlling the temperature of the heat-treated slurry to provide a slurry having a temperature from about 105.degree. to about 140.degree.F. for enzymatic hydrolysis,

3. subjecting the resulting slurry to enzymatic hydrolysis conditions, including the action of neutral protease at a temperature of about 105.degree. to 150.degree.F. for a residence time of about 30 to 150 minutes, to produce water-soluble polypeptides,

4. adjusting the pH of the resulting slurry to about 3.0 to 4.0 to provide acidic conditions comparable to the pH desired for the protein beverage and to inactivate the enzyme, and

5. removing undissolved solids from the slurry to leave an acidic, polypeptide-containing mother liquor containing about 4 to 8 weight percent of dissolved solids; the improvement comprising

6. evaporating from said mother liquor having a pH from about 3.0 to 4.0 substantially all of those off-flavor ingredients in the polypeptide product which boil below the boiling point of water, said evaporation being effected at a temperature sufficient to separate the ingredients from the mother liquor and over a period of time sufficient to evaporate the ingredients but insufficient to effect substantial degradation of the polypeptide matter in the mother liquor, and

7. formulating the evaporation residue into an acidic, carbonated beverage.





Generate Collection

L10: Entry 6 of 16

File: USPT

Jul 12, 1988

DOCUMENT-IDENTIFIER: US 4757007 A

TITLE: Process for preparing hydrolyzed products of soy proteinAbstract Paragraph Left (1):

Two kinds of hydrolyzed protein having different characteristics respectively are obtained by hydrolyzing soy protein with protease and separating the mixture of hydrolyzed products using their solubilities in a 5% trichloro acetic acid aqueous solution as the guidance of the separation.

Abstract Paragraph Left (2):

The hydrolyzed protein of the low solubility possesses excellent emulsifying properties, and the one of the high solubility possesses excellent foaming properties.

Brief Summary Paragraph Right (2):

This invention relates to a process for preparing two kinds of hydrolyzed products of soy protein by separating the products after partially hydrolyzing soy protein with a protease.

Brief Summary Paragraph Right (5):

Up to the present, it is known that the emulsifying properties or foaming properties of soy protein are improved by hydrolyzing it partially with protease (see "Studies on enzyme modified proteins as foaming agents: Effect of structure on foam stability" T. Horiuchi et al., Food Chem., 3, 35(1978); "Studies on the emulsifying properties of soybean proteins: Part II. Effect of partial hydrolysis" H. Aoki et al., Nippon Shokuhin Kogyo Gakkaishi, 23, 26(1976); and "Modification of functional properties of soy proteins by proteolytic enzyme treatment", G. Puski, Cereal Chem., 52, 655(1975)).

Brief Summary Paragraph Right (6):

But a process that is able to prepare simultaneously a foaming agent and an emulsifying agent by hydrolyzing soy protein with protease and separating the hydrolyzed products subsequently has not been found yet.

Brief Summary Paragraph Right (7):

It has been found that both the hydrolyzed protein possessing emulsifying properties and the other hydrolyzed protein possessing foaming properties can be obtained simultaneously from the hydrolyzed products by using the solubility of them by 5% aqueous solution of trichloroacetic acid (hereinafter referred to as "a 5% TCA solubilization by weight") as the guidance of separation.

Brief Summary Paragraph Right (8):

In accordance with this invention, there is provided a process for the preparation of hydrolyzed products of soy protein which comprises partially hydrolyzing soy protein with protease, then separating the resulting hydrolyzed products by using the 5% TCA solubilization by weight into two parts wherein one part has a solubilization of 10-40% by weight and the other part has a solubilization of 70% or more by weight. The former is useful for emulsifying agent, and the latter is useful for foaming agent.

Brief Summary Paragraph Right (9):

In accordance with this invention, protein of soybean is used for raw material. Soy proteins treated by heat or with alcohol are used for increasing the efficiency of hydrolysis and/or for improving the flavor of the final products.

Brief Summary Paragraph Right (10):

As the protease being used for hydrolysis in accordance with this invention, there can be employed, for example, pepsin, bromelain, papain and the like which are obtained from animals, plants and bacterium.

Brief Summary Paragraph Right (11):

The partial hydrolysis is conducted by dispersing soy protein into water, adjusting the pH and the temperature to the optimum pH and the temperature of the enzyme being used, and adding the enzyme subsequently.

Brief Summary Paragraph Right (12):

The reaction time of partial hydrolysis should be long enough to hydrolyze soy protein with protease until two kinds of hydrolyzed products are formed wherein one is a low molecular weight part having high foaming capacity and wherein the other one is a high molecular weight part having high emulsifying capacity.

Brief Summary Paragraph Right (14):

The hydrolysis is terminated by inactivating the enzyme by heating at 75.degree. C. for more than 5 minutes.

Brief Summary Paragraph Right (15):

The hydrolyzed products thus obtained are separated into two parts which have the 5% TCA solubilizations by weight expected.

Brief Summary Paragraph Right (17):

The first method comprises adjusting the pH of the hydrolyzed solution to pH6.8-7.0, optionally removing insoluble matter, and adjusting the pH of the solution to pH2.5-5 to precipitate, then separating the supernatant and the precipitate by centrifugation. The pH of the solution at the separating operation by precipitating should be decided to be the optimum pH between pH2.5-5 to separate out the precipitate having the 5% TCA solubilization by weight of 10-40% from the supernatant having the 5% TCA solubilization by weight of 70% or more in its dried powdery state, paying attention to the time of hydrolysis. Contrary, to what would be expected when precipitating beyond this pH range, the hydrolyzed products having the expected solubilization rates can not be obtained.

Brief Summary Paragraph Right (19):

Usually, treating the solution with a commercial ultrafiltration membrane of 15,000-20,000 molecular weight cut off, the filtrate passed through the membrane contains the hydrolyzed protein which has the 5% TCA solubilization by weight of 70% or more, and the residual solution after completing the filtration contains the hydrolyzed protein which has the 5% TCA solubilization by weight of 10-40%.

Brief Summary Paragraph Right (20):

The thus obtained hydrolyzed proteins having the 5% TCA solubilization by weight of 10-40% and 70% or more are used as such solution, or used in a powdered state after being dried.

Brief Summary Paragraph Right (21):

In accordance with this invention, the hydrolyzed soy protein having the 5% TCA solubilization by weight of 10-40% possesses high emulsifying capacity and excellent emulsifying stability, and the one having the 5% TCA solubilization by weight of 70% or more possesses high foaming capacity and excellent foaming stability.

Brief Summary Paragraph Right (22):

A hydrolyzed soy protein having the 5% TCA solubilization by weight beyond the range of 10-40% lacks emulsifying capacity and can not work as an emulsifying agent. On the other hand, a hydrolyzed soy protein having the 5% TCA solubilization by weight less than 70% lacks good foaming capacity and can not work as foaming agent.

Brief Summary Paragraph Right (23):

As is apparent from the above, this invention provides the process of partially hydrolyzing raw material of soy protein with a protease, simultaneously separating the hydrolyzed products into two parts which have emulsifying properties or foaming properties respectively using their solubilities by weight in 5% TCA as the guidance of separation, and the process may be conducted easily.

Detailed Description Paragraph Right (18):

1 kg of soy protein isolate was dispersed in ten times amount of water, then the pH of the mixture was adjusted to pH7.0. 10 g of papain (by Novo Industry Incorporated) was added to the mixture, and hydrolysis of the protein was carried out for 6 hours. Then, the mixture was heated at 75.degree. C. for 20 minutes to inactivate the protease. The dried content of the hydrolyzed solution had a 5% TCA solubilization rate of 63.8%.

Detailed Description Paragraph Right (20):

The precipitate separated above is dispersed in water, neutralized to pH6.8 with NaOH and freeze dried to obtain 540 g of the product (hereinafter referred to as "Hydrolyzed Protein I"). Hydrolyzed Protein I had a 5% TCA solubilization by weight of 30.2%.

Detailed Description Paragraph Right (21):

The supernatant is neutralized to pH6.8 with NaOH too, and freeze dried to obtain 420 g the product (hereinafter referred to as

"Hydrolyzed Protein II").

Detailed Description Paragraph Right (22):

Hydrolyzed Protein II had a 5% TCA solubilization by weight of 80.4%. The emulsifying properties and the foaming properties of those hydrolyzed products are shown in Table I. Hydrolyzed Protein I possessed high emulsifying capacity of 360 as shown in the table, and excellent emulsifying stability. Hydrolyzed Protein II possessed high foaming capacity of 900 and excellent foaming stability of 79% respectively.

Detailed Description Paragraph Right (23):

On the other hand, the emulsifying capacity of the control of hydrolyzed proteins having the 5% TCA solubilization by weight of 63.8% obtained by directly freeze drying the hydrolyzed solution before being separated was inferior to that of the example of this invention as it was 260, and the emulsifying stability was not good. The foaming capacity and the foaming stability of the control are inferior to those of the example as they were 580 and 60% respectively.

Detailed Description Paragraph Right (24):

Defatted soy flour was dispersed in ten times weight of water, then the pH of the mixture was adjusted to pH7.0. The mixture was heated at 50.degree. C. under stirring for 30 minutes, then insoluble substance was rejected with centrifugation.

Detailed Description Paragraph Right (25):

Ethanol was added to the aqueous supernatant until 65%(w/w) alcoholic concentration to precipitate protein. The precipitate protein was separated from the mixture by centrifugation and dried. 500 g of the dried protein was dispersed in twenty times weight of water and acidified to pH1.8 by HCl. 2 g of pepsin (by Amano Pharmaceutical Co., Ltd.) was added to the mixture, then hydrolysis was conducted at 55.degree. C. for 4 hours. Then the mixture was heated at 75.degree. C. for 20 minutes to inactivate enzymes. The hydrolyzed solution was neutralized to pH6.8 by NaOH and subjected to separation with an ultrafiltration membrane. An ultrafiltration membrane (IRIS3038 by Rhone-Poulenc Co., Ltd.) whose molecular weight cut off is 15,000-20,000 was used for this separation.

Detailed Description Paragraph Right (26):

After this filtration, the residual solution was freeze dried to obtain 220 g of product (hereinafter referred to as Hydrolyzed Protein III) having the 5% TCA solubilization by weight of 20.7%. The filtrate was freeze dried to obtain 230 g of product (hereinafter referred to as Hydrolyzed Protein IV) having the 5% TCA solubilization by weight of 86.1%.

Detailed Description Paragraph Right (27):

The emulsifying properties and the foaming properties of the hydrolyzed products thus obtained are shown in Table II. Hydrolyzed Protein III possessed high emulsifying capacity of 340 as shown in the table and excellent emulsifying stability. Hydrolyzed Protein IV possessed high foaming capacity of 980 and excellent foaming stability of 86% respectively.

Detailed Description Paragraph Table (1):

| TABLE I       | 5% TCA           | <u>Hydrolyzed</u> | <u>Hydrolyzed</u> | Solubilization (wt %)  | Control             |
|---------------|------------------|-------------------|-------------------|------------------------|---------------------|
| Protein I     | Protein II       |                   |                   | emulsifying Properties | 63.8 30.2 80.4      |
| ##STR1##      | 260 360          | No emulsification | Emulsifying       | Not good               | Good --             |
| Capacity (ml) | Foaming 60 54 79 | Stability         |                   |                        |                     |
|               |                  |                   |                   | Stability              | Foaming 580 400 900 |

Detailed Description Paragraph Table (2):

| TABLE II | <u>Hydrolyzed</u>         | <u>Hydrolyzed</u>         | Protein III              | Protein IV    |
|----------|---------------------------|---------------------------|--------------------------|---------------|
|          | 5% TCA                    | 20.7 86.1                 | Solubilization (wt %)    | emulsifying   |
|          | emulsification Properties | Capacity g of soybean oil | 1 g of sample            | Emulsifying   |
|          |                           |                           | Good --                  | Stability     |
|          |                           |                           | Foaming                  | Foaming       |
|          |                           |                           | 420 980                  |               |
|          |                           |                           | Properties Capacity (ml) | Foaming 52 86 |
|          |                           |                           | Stability (%)            |               |

CLAIMS:

1. A process for preparing hydrolyzed products of soy protein which comprises

(a) partially hydrolyzing soy protein with a protease to obtain a solution containing two parts of said hydrolyzed products having differing solubilities in 5% trichloroacetic acid aqueous solution, wherein one of said two parts has a solubility of 10-40% by weight in 5% trichloroacetic acid and the other of said two parts has a solubility of 70% or more by weight in 5% trichloroacetic acid, and

(b) separating said two parts from each other by steps consisting essentially of neutralizing the solution containing two parts of said hydrolyzed products to pH 6.8-7.0, removing insoluble matter from said solution, and separating said hydrolyzed products

into said two parts with an ultrafiltration membrane.

2. A process for preparing hydrolyzed products of soy protein which comprises

(a) partially hydrolyzing soy protein with a protease to obtain a solution containing two parts of said hydrolyzed products having differing solubilities in 5% trichloroacetic acid aqueous solution, wherein one of said two parts has a solubility of 10-40% by weight in 5% trichloroacetic acid and the other of said two parts has a solubility of 70% or more by weight in 5% trichloroacetic acid, and

(b) separating said two parts from each other by steps consisting essentially of neutralizing the solution containing two parts of said hydrolyzed products to pH 6.8-7.0, removing insoluble matter from said solution, precipitating one of said two parts of said hydrolyzed products by acidifying said solution to pH 2.5-5 to form a supernatant and precipitate, and separating the supernatant and the precipitate.

4. The process of claim 1 which comprises heating the soy protein or precipitating the soy protein with alcohol before hydrolysis.

6. The process of claim 5 wherein the protease is papain.

8. The process of claim 2 which comprises heating the soy protein or precipitating the soy protein with alcohol before hydrolysis.

9. The process of claim 3 which comprises heating the soy protein or precipitating the soy protein with alcohol before hydrolysis.

11. The process of claim 10 wherein the protease is papain.

14. The process of claim 13 wherein the protease is papain.

17. The process of claim 16 wherein the protease is papain.

## End of Result Set

**Generate Collection**

L10: Entry 16 of 16

File: USPT

Aug 21, 1973

DOCUMENT-IDENTIFIER: US 3753728 A

TITLE: PROCESS FOR PRODUCTION OF SOY-CONTAINING BREAKFAST CEREALS

Abstract Paragraph Left (1):

Process for making ready-to-eat breakfast cereal containing soy protein. Soy protein is made more palatable by subjecting it to a partial hydrolysis reaction in the presence of a specific mixture of proteolytic enzymes. A mixture of proteolytic enzyme papain and at least one other proteolytic enzyme has the effect of more efficiently causing the partial hydrolysis of soy protein than does a single enzyme at the same addition level.

Brief Summary Paragraph Right (5):

Commonly assigned, copending applications Ser. No. 50,980, Production of Ready-to-Eat Breakfast Cereals Containing Soy Flour by Alexander L Liepa, Ser. No. 50,925, High Protein Ready-to-eat Breakfast Cereals Containing Soy Concentrate by William T. Bedenk, and Ser. No. 50,924, High Protein Ready-to-Eat Breakfast Cereals Containing Soy Isolate by William T. Bedenk, all contain disclosures as to the treatment of their respective soy protein sources to make them more compatible. The treatment comprises mixing 50 to 80 percent water by weight of the total mixture with the protein source and adding 25 ppm - 2500 ppm of a proteolytic enzyme or enzymes based on the soy protein source. This mixture is held at 80.degree.F to 160.degree.F for 1 minute to 120 minutes to cause a partial hydrolysis of the soy protein source. Such a treatment unexpectedly improves the flavor, crispness retention, tenderness, and puffability of a cereal product containing the soy protein to such an extent that the soy protein can form a major portion of the end product.

Brief Summary Paragraph Right (6):

In accord with this invention a cold cereal product is produced containing a soy protein source which has been partially hydrolyzed in a more efficient manner than known heretofore. The total proteolytic enzyme level needed to obtain a partial hydrolysis to the degree desired is reduced to a level which prior to this invention was ineffective.

Brief Summary Paragraph Right (8):

It is a further object of this invention to produce a soy protein-containing high protein content ready-to-eat breakfast cereal by a process whereby the soy protein is partially hydrolyzed to the degree desired in a very efficient manner.

Brief Summary Paragraph Right (9):

It is a further object of this invention to produce a soy protein-containing high protein content ready-to-eat breakfast cereal by a process whereby the soy protein is partially hydrolyzed in the presence of a specific mixture of proteolytic enzymes, which specific mixture causes a more efficient partial hydrolysis than does a single enzyme at the same addition level.

Brief Summary Paragraph Right (10):

More specifically, it is an object of the present invention to produce a high protein content ready-to-eat cereal by a process wherein the protein source is partially hydrolyzed in the presence of papain and at least one other proteolytic enzyme.

Brief Summary Paragraph Right (12):

Briefly stated, this invention concerns the production of highly palatable and nutritive breakfast cereal products. More specifically, the cold cereal products of this invention comprise a soy protein source and a cooked cereal grain in proportions such that the total protein content of the breakfast cereal is at least 20 percent. Such a product is produced by mixing water with a soy protein source, papain and at least one other proteolytic enzyme. The above mixture is then exposed to an elevated temperature for a length of time sufficient to cause a partial hydrolysis of the soy protein source and then further processed into the desired end product.

Brief Summary Paragraph Right (13):

In the present invention there is produced a protein enriched cold cereal product that is produced in any shape or form desired

such as shredded, puffed, crumbled, biscuit, granule, flaked and the like. Soy protein is used as the major source of protein. As used herein, soy protein is generic to soy flour, soy protein concentrate, and soy protein isolate and will be used in the description to follow. All three protein sources are commercially available and the use of them in the present invention can be done interchangeably. Any deviations in the processing of the three protein sources will be noted in the description to follow. The soy protein sources useful in this invention are defatted and refined soybeans. Soy flour contains 40 up to 70 percent protein, soy protein concentrate contains 70 up to 90 percent protein and soy protein isolate contains 90 up to 100 percent protein.

Brief Summary Paragraph Right (14):

In accord with this invention soy protein is made more palatable by subjecting it to a partial hydrolysis. The partial hydrolysis is accomplished by including in the reaction mixture the proteolytic enzyme papain plus at least one other proteolytic enzyme. The mixture of the above enzymes is able to promote a partial hydrolysis while an equal amount of a single enzyme does not cause a partial hydrolysis to the same degree. The degree of partial hydrolysis of the soy protein is important because it has a direct effect on the soy-containing end product's taste. That is, a different degree of partial hydrolysis in two protein sources will result in different eating qualities of two cereal products containing the respective soy protein source. The greater the degree of partial hydrolysis of the soy protein source the more tender will be a cold cereal product containing that partially hydrolyzed soy protein and, hence, more desirable up to a point, i.e., a too tender product is also undesirable to the average consumer. As taught herein, the exact degree of partial hydrolysis desired is obtained in a very efficient manner when the soy protein and water are reacted in the presence of papain and at least one other proteolytic enzyme at the conditions set out hereinafter. The reaction is efficient in the sense a lower total proteolytic enzyme level promotes the partial hydrolysis to the exact degree desired when the specific enzyme mixture papain and at least one other proteolytic enzyme is included in the reaction mixture as opposed to other enzyme mixtures or single enzymes at the same reaction conditions.

Brief Summary Paragraph Right (15):

After the proper degree of partial hydrolysis of the soy protein source has occurred, it is further processed into the final product. A preferred method comprises extruding the partially hydrolyzed soy protein source into strands of a relatively small cross-sectional area, pelletizing the strands, flaking and puffing or immediately puffing after pelletizing. Additional steps such as toasting or coating can be added to further enhance the product's taste and/or appearance.

Brief Summary Paragraph Right (16):

In a preferred embodiment of this invention a gelatinized cereal grain is added to a partially hydrolyzed soy protein in proportions such that the total protein content of the end product is at least 20 percent. This mixture is further processed into a final form suitable for human consumption in the manner above described for the soy source alone.

Brief Summary Paragraph Right (17):

By the process of the present invention the soy protein is made more palatable by forming a mixture of the soy protein source, water, the proteolytic enzyme papain, and at least one other proteolytic enzyme. Quite unexpectedly, the above specific mixture of proteolytic enzymes promotes a partial hydrolysis to the degree desired while an equal level of either papain alone, another proteolytic enzyme or a mixture of other proteolytic enzymes under the same reaction conditions does not result in the same degree of partial hydrolysis. Only at levels of proteolytic enzymes substantially greater than the total level of papain and at least one other proteolytic enzyme is there obtained the same degree of partial hydrolysis under the same conditions. In that proteolytic enzymes are relatively expensive it is imperative that as low a level of proteolytic enzyme as possible commensurate with the proper degree of partial hydrolysis be used.

Brief Summary Paragraph Right (18):

No hydrolysis of the soy protein or only a partial hydrolysis less than that achieved by following the reaction conditions of this invention gives an unacceptable tasting product as well as a poorly processable ingredient. A degree of hydrolysis of the soy protein in excess of that experienced under the conditions set out hereinafter results in a product having an unacceptable taste. Only when the soy protein is partially hydrolyzed to the degree taught herein and further processed to an end product is there obtained an acceptable soy-containing high protein ready-to-eat breakfast cereal.

Brief Summary Paragraph Right (19):

The proteolytic enzymes useful in the present invention in conjunction with papain are selected from any of several known proteolytic enzymes or mixtures thereof extracted from animal, plant, fungal, or microbial sources. A primary consideration in the enzyme or enzyme mixture used is that it must not contribute a significantly objectionable flavor or odor to the final product. Some examples of proteolytic enzymes found effective in the soy protein partial hydrolysis step that can be used with the papain are pepsin, bromelin, ficin, alcalase, maxitase, thermoase, pronase, and mixtures thereof.

Brief Summary Paragraph Right (20):

In this invention, 15 ppm - 2500 ppm of papain and 5 ppm- 2,500 ppm of at least one other proteolytic enzyme by weight of the soy protein is sufficient to cause the desired degree of partial hydrolysis when the reaction mixture is exposed to a temperature of

80.degree.F to 160.degree.F for 1 minute to 120 minutes. The preferred levels of enzymes are 100 ppm - 300 ppm of the papain and 100 ppm - 300 ppm of at least one other proteolytic enzyme based on the weight of the protein. Temperatures of 120.degree.F to 130.degree.F and times of 1 minute to 5 minutes are preferred.

Brief Summary Paragraph Right (21):

The amount of water needed for the partial hydrolysis reaction is basically determined by apparatus limitations. That is, the lower limit of water is determined by the capability of the mixing equipment. The lower the level of water the more viscous will be the resultant mixture. On the other hand, an excessive amount of water in the partial hydrolysis reaction would necessitate additional work in reducing the water level in subsequent processing steps. The preferred level of water is 50 to 80 percent based on the total weight of the mixture. The most preferred level is 55 to 60 percent based on the total weight of the mixture.

Brief Summary Paragraph Right (22):

Under the above conditions the soy protein source is partially hydrolyzed to the extent that a cold cereal product containing the soy protein is acceptable with regard to taste, tenderness, crispness retention, and processability.

Brief Summary Paragraph Right (23):

An equal level of a single enzyme, e.g., papain or any other enzyme, and the same reaction conditions produces an end product which is not as acceptable with regard to eating quality and processability as the product produced in accord with this invention, thereby indicating that the soy protein source has not been partially hydrolyzed to the degree needed. That such an effect would be obtained with the specific mixture of proteolytic enzymes, papain and another enzyme as opposed to single enzymes or any other mixture was quite unexpected.

Brief Summary Paragraph Right (24):

The production of a cold breakfast cereal containing the partially hydrolyzed soy protein is done by various general procedures used for making cold cereal products and depends in large part on the desired form, type, or condition of the final product. Typically the partially hydrolyzed soy protein is extruded into strands of a relatively small cross-sectional area and thereafter sliced into small lengths thereby forming small pellet-like particles. These pellet-like particles are partially dried, if necessary, and formed into flakes. The flakes are then subjected to a puffing operation to transform them into less dense, more porous, and tender flakes. Toasting and/or a coating operation may be employed to enhance the color and/or flavor of the resultant protein fortified cereal product. Alternatively, instead of producing a flake-like product, the flaking step can be omitted with a puffed pellet-shaped product being the result.

Brief Summary Paragraph Right (25):

In the preferred method of transforming the partially hydrolyzed soy protein-water dough into the finished product, the first step is to extrude the dough into strands. An extruder has the effect of mixing the ingredients even more intimately and of forming the dough into a shape easier to handle and more adaptable for existing equipment. Relatively low pressures in the extruder are sufficient for this operation. Pressures within the range of 500 p.s.i.g. to 1,000 p.s.i.g. are preferred. Lower pressures can be used but should preferably be avoided since less of a mixing action in the extruder results from the low compressive forces associated with low pressures. Pressures higher than 1,000 p.s.i.g. exert little extra benefits and for this reason are avoided. Temperatures employed in the extrusion process are not a critical feature but do have some effect on the handling characteristics of the extrudant, such as stickiness and body. Temperatures falling within the range of 140.degree.F to 200.degree.F have been found to be satisfactory.

Brief Summary Paragraph Right (30):

Breakfast cereals obtain the desired flake structure by a process known as puffing. Puffing of the flake is also quite important in that it enhances the flake's crispness and tenderness. Cereal flakes containing untreated soy protein are difficult to puff but, unexpectedly, soy protein when partially hydrolyzed in the manner heretofore described does not act as a hindrance on puffing but rather actually improves puffability. This factor is of importance in that the more porous type flakes have a tendency to be more tender than the less porous or less puffed flake. Additionally, soy flavor is diminished even more in the better puffed of two soy-containing flakes. Basically a cereal is puffed by causing trapped moisture in the flake to expand very rapidly from the liquid state to the vapor phase. Rapid heating or a rapid decrease in the pressure are the methods commonly used to convert dense hard flakes into the more palatable porous tender flake. Both methods are well known and are commonly used throughout the industry. Gun puffing is an example of the principle of a rapid decrease in pressure. In this process the cereal flakes are first heated under high pressure and then the pressure is rapidly released to achieve the puffing effect. The process disclosed in U.S. Pat. No. 3,253,533 is an example of a rapid heating puffing method. Commonly assigned copending application Ser. No. 76771, Apparatus and Process of Puffing, by William T. Bedenk and Lawrence Gabel, also discloses a rapid heating puffing method.

Brief Summary Paragraph Right (35):

In accordance with another and preferred aspect of the present invention, a cereal grain selected from the group consisting of corn, wheat, rice, barley, oats, and mixtures thereof, is admixed with the partially hydrolyzed soy protein and thereafter processed

to form a composite final product that still has a high protein content, i.e., greater than 20 percent. As with the cereal product made from soy protein alone forming the structure of the product, the cereal product comprising treated soy protein and a cereal grain is produced by general processes of cereal manufacture depending on the desired form, type, or condition of the final product.

Brief Summary Paragraph Right (36):

In the preferred process the additive cereal grain is separately cooked or gelatinized and then mixed with the partially hydrolyzed soy protein to form a dough. This dough is then processed in accord with the preferred process above described with respect to the all soy protein cereal product. That is, the dough is extruded, pelletized, dried if necessary, and puffed. Alternatively, the pellets are flaked prior to the puffing operation to form a flaked product. Toasting and/or a coating operation may be added to the process.

Brief Summary Paragraph Right (39):

The gelatinized cereal grain can, at this point, be added to the previously partially hydrolyzed soy protein and further processed to produce the cold cereal product. Preferably, though, the water content of the gelatinized cereal grain is reduced prior to mixing with the partially hydrolyzed protein source. This additional operation is preferred at this point in the process so that subsequent handling and processing operations proceed more smoothly. Excessive moisture levels in the mixture cause subsequently formed individual cereal forms or shapes to lack body or be overly soft and difficult to handle. The amount of moisture present in the cooked cereal grain at the time of addition to the treated soy protein mixture must be relatively low because water still present from the partial hydrolysis of soy protein will contribute significantly to the total moisture content of the mixture. It is preferred that 15 to 30 percent water be present in the soy protein-cooked grain mixture when the product is being made by the preferred method. Accordingly, to reach the lower water levels often desired, less than 5 percent water must be present in the cooked cereal grain prior to mixing with the soy flour. If the cereal is gelatinized by the continuous extrusion method under pressure, as in the preferred cooking method, the resultant extrudant may flash dry and thereafter contain less than 5 percent water and as such would not need the additional drying operation.

Brief Summary Paragraph Right (41):

The cooked cereal grain can now be combined with the partially hydrolyzed soy protein or, optionally, given one more treatment to improve its processability. That is, the cooked cereal grain can be vigorously milled to increase its free starch content. In this regard reference is made to commonly assigned copending application Ser. No. 76990, Production of Puffed Ready-to-Eat Cereal Products, by William T. Bedenk and John W. Mitchell.

Brief Summary Paragraph Right (42):

The cooked cereal grain is now combined with the partially hydrolyzed soy protein to form a dough and thereafter processed in the manner heretofore described with respect to the treated soy protein alone. Corn, rice, oats, and wheat all contain relatively low protein contents that must be accounted for when determining the total protein content of a soy flour-cereal grain cereal product. The approximate protein contents of corn, rice, oats, and wheat are 9 percent, 7 percent, 14 percent, and 12 percent, respectively. The exact protein content of a cereal grain and of the soy protein are determined by methods well known to those skilled in the art. The partially hydrolyzed soy protein and gelatinized cereal grain are combined together in proportions such that the end product has a protein content of at least 20 percent.

Brief Summary Paragraph Right (43):

It should be understood that the soy protein partially hydrolyzed as taught herein can be further processed into a ready-to-eat breakfast cereal by processes in addition to the previously described extruding, pelletizing, flaking, and/or puffing process. For instance, one especially preferred method is the "extrusion puffing" method. In this method the partially hydrolyzed soy protein is mixed with other ingredients that go to making up the desired cold cereal composition, e.g., sugar, salt, gelatinized cereal grain, and thereafter fed into an extruder-puffer. Under operating conditions of 500 psig-1,000 psig at the puffing end, temperatures of 280.degree. to 320.degree. F and a speed of rotation of the extruder screw of 120-300 rpm, a very satisfactory puffed cold cereal product is obtained.

Detailed Description Paragraph Right (4):

A cold cereal product is made by the same formulation and process steps and conditions of Example 1 with the exception that 0.24 papain (363 ppm) is substituted for the papain-alcalase mixture above. The same panel tested the resultant product in the same manner as Example 1 and rated it as follows:

Detailed Description Paragraph Right (5):

A cold cereal product is made by the same formulation and process steps and conditions of Example 1 with the exception that 0.32 grams papain (484 ppm) is substituted for the papain-alcalase mixture. The same panel rated the resultant product on the basis of the above described test as follows:



Detailed Description Paragraph Right (6):

A comparison of the ratings of the product of Comparative Test B and the product of Comparative Test A shows that even though a greater level of papain (33 percent greater) is used, the resultant product is very similar in regard to tenderness.

Detailed Description Paragraph Right (7):

A comparison of the ratings of the product of Comparative Test B with the ratings of the product of Example 1 shows that even an increased level of a single proteolytic enzyme (484 ppm for this comparative test vs. a total enzyme level of 363 ppm for Example 1) does not give as tender a product as does a papain and other proteolytic enzyme mixture. Since enzyme level affects the degree of partial hydrolysis, it can be seen that a substantially greater level of a single proteolytic enzyme would be needed to give the same degree of partial hydrolysis and hence same tenderness rating as obtained by the specific mixtures of this invention.

Detailed Description Paragraph Left (1):

A comparison of the two tests shows that at all time intervals the first product, i.e., the formulation of Example 1 containing papain-alcalse, is more tender than the product of this comparative test containing papain at the same total proteolytic enzyme level.

Detailed Description Paragraph Table (1):

Soy isolate 662 grams Water 830 grams Papain 0.16 grams (242 ppm) Alcalase 0.08 grams (121 ppm)

## CLAIMS:

1. In a process for the production of a ready-to-eat breakfast cereal containing soy protein and having a protein content of at least 20 percent, said process including the steps of preparing a soy protein containing cereal dough, shaping said dough, and puffing said dough, the improvement which comprises: reacting the soy protein with water for about 1 minute to about 120 minutes at about 80.degree.F to about 160.degree.F in the presence of from about 15 ppm to about 2,500 ppm of papain and from about 5 ppm to about 2,500 ppm of at least one other proteolytic enzyme, thereby causing partial hydrolysis of the soy protein to a degree that results in a product which is easily processed and which has an acceptable taste.

4. A process for making a palatable ready-to-eat breakfast cereal having a protein content of at least 20 percent comprising:

a. reacting soy protein with water in the presence of from about 15 ppm to about 2,500 ppm of papain and from about 5 ppm to about 2,500 ppm of at least one other proteolytic enzyme for 1 minute to 120 minutes at 80.degree.F to 160.degree.F, thereby causing a partial hydrolysis of the soy protein;

b. extruding the partially hydrolyzed soy protein into strands of a desired shape;

c. slicing the strands into pellet-like particles; and

d. puffing the pellet-like particles to form the ready-to-eat breakfast cereal.

5. The process of claim 4 further comprising blending a gelatinized cereal grain with the partially hydrolyzed soy protein prior to the extruding step.

9. The process of claim 4 wherein the papain and at least one other proteolytic enzyme are each present in an amount ranging from 100 ppm to 300 ppm by weight of the soy protein.

11. A process for making a palatable ready-to-eat breakfast cereal having a protein content of at least 20 percent comprising:

a. reacting soy protein with water in the presence of from about 15 ppm to about 2500 ppm of papain and from about 5 ppm to about 2500 ppm of another proteolytic enzyme for 1 minute to 120 minutes at 80.degree.F to 160.degree.F, thereby causing a partial hydrolysis of the soy protein; and

b. extruding-puffing the partially hydrolyzed soy protein to form the ready-to-eat breakfast cereal.

12. The process of claim 11 wherein 15 ppm-2500 ppm of the papain and 5 ppm-2500 ppm of at least one other proteolytic enzyme by weight of the soy protein are present in the reaction.

13. The process of claim 11 wherein the partially hydrolyzed soy protein is extruded-puffed under a pressure of 500 psig - 1000 psig and a temperature of 280.degree.F - 320.degree.F.

14. The process of claim 13 further comprising blending a gelatinized cereal grain with the partially hydrolyzed soy protein prior to the extruding-puffing step.